TLC/SERS coupling : sensibility and selectivity gains as illustrated with quinquina alcaloids.

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Using Raman spectrometry for molecular identification of chromatographically separated species has been proposed few years ago and has become a subject of a large number of published reports [refs]. The limitations of conventional Raman (fluorescence interference and low sensitivity) have lead these applications to using Fourrier-transform (FT) Raman in near-infrared and SERS (surface-enhanced Raman spectrometry; Fleishmann et coll., 1974) techniques. Compared to FT-Raman, the SERS method is known to provide not only fluorescence reducing but also a sensitivity gain of up to a billion-fold scale.

In the present study, we have evaluated comparatively the FT-Raman and FT-SERS (silver colloids) tools when used to characterize the TLC patterns from quinquina alcaloids (quinine, quinidine, cinchonidine, cinchonine) – molecules of well-known pharmacological interest, namely in paludism treatment.

Prior to TLC experiments, the FT-Raman and FT-SERS spectra have been recorded for these four compounds in powder or in solution. The spectra provided a distinct patterns for each quinine derivative (including their respective polymorphs). The temptative assignment of the major Raman bands of these compound have been proposed from the experimental data.

Looking for the best efficacy of the TLC-SERS coupling, both chromatographic and spectroscopic experimental protocols were optimized. Indeed, the choice of the TLC plates and of the revealing agents as well as the silver colloids preparation/ manipulation were crucial at both qualitative and quantitave level.

Compared to FT-Raman, the FT-SERS approach provided the selectivity and sensitivity gain of at least three orders of magnitude. With FT-SERS, the alcaloid detection was still enabled down to nano-gramms, while with FT-Raman the detection limits were over microgramms. Within these limit conditions, it was possible to distinguish the methoxy- substitution, while the identification of the respective stereo-isomers required about ten times greater sample concentration. The results have been validated with mixtures of the four pure compounds as well as with natural extracts from quinquina.